

This is a repository copy of *S-Adenosyl Methionine Cofactor Modifications Enhance the Biocatalytic Repertoire of Small Molecule C-Alkylation*.

White Rose Research Online URL for this paper:

<https://eprints.whiterose.ac.uk/153066/>

Version: Accepted Version

---

**Article:**

Grogan, Gideon James orcid.org/0000-0003-1383-7056, McKean, Iain, Sadler, Joanna et al. (5 more authors) (2019) S-Adenosyl Methionine Cofactor Modifications Enhance the Biocatalytic Repertoire of Small Molecule C-Alkylation. *Angewandte Chemie International Edition*. pp. 1-7. ISSN 1433-7851

<https://doi.org/10.1002/anie.201908681>

---

**Reuse**

Items deposited in White Rose Research Online are protected by copyright, with all rights reserved unless indicated otherwise. They may be downloaded and/or printed for private study, or other acts as permitted by national copyright laws. The publisher or other rights holders may allow further reproduction and re-use of the full text version. This is indicated by the licence information on the White Rose Research Online record for the item.

**Takedown**

If you consider content in White Rose Research Online to be in breach of UK law, please notify us by emailing [eprints@whiterose.ac.uk](mailto:eprints@whiterose.ac.uk) including the URL of the record and the reason for the withdrawal request.

# S-Adenosyl Methionine Cofactor Modifications Enhance the Biocatalytic Repertoire of Small Molecule C-Alkylation

Iain J. W. McKean,<sup>[a,b]</sup> Joanna C. Sadler,<sup>[a,c]</sup> Anibal Cuetos,<sup>[d]</sup> Amina Frese,<sup>[d]</sup> Luke D. Humphreys,<sup>[c]</sup> Gideon Grogan,<sup>\*[d]</sup> Paul A. Hoskisson,<sup>\*[b]</sup> Glenn A. Burley<sup>\*[a]</sup>

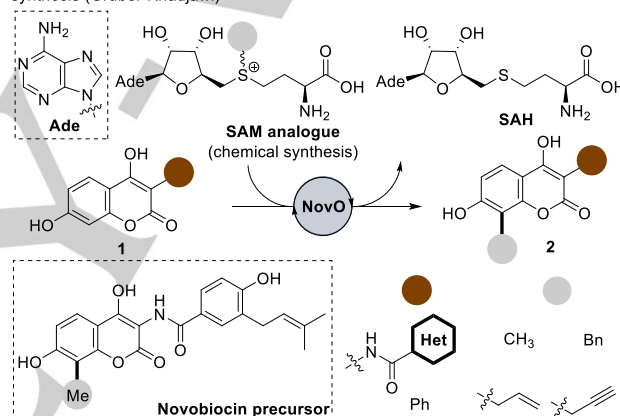
**Abstract:** A tandem enzymatic strategy to enhance the scope of C-alkylation of small molecules *via* the *in situ* formation of S-adenosyl methionine (SAM) cofactor analogues is described. A solvent-exposed channel present in the SAM-forming enzyme SalL tolerates 5'-chloro-5'-deoxyadenosine (CIDA) analogues modified at the 2-position of the adenine nucleobase. Coupling SalL-catalyzed cofactor production with C-(m)ethyl transfer to coumarin substrates catalyzed by the methyltransferase (MTase) NovO forms C-(m)ethylated coumarins in superior yield and greater substrate scope relative to that obtained using cofactors lacking nucleobase modifications. Establishing the molecular determinants which influence C-alkylation provides the basis to develop a late-stage enzymatic platform for the preparation of high value small molecules.

Regiospecific methylation is an essential process used in Nature to modulate biological function.<sup>[1, 2]</sup> From an industrial perspective, methylation of small molecules is a powerful strategy to fine-tune their physicochemical properties and enhance overall drug potency.<sup>[3]</sup> In order to fully exploit this 'magic methyl effect' across the pharmaceutical and biotechnology sectors,<sup>[4, 5]</sup> robust methods are required to precisely methylate - and indeed alkylate - substrates in an environmentally benign manner.<sup>[5-7]</sup>

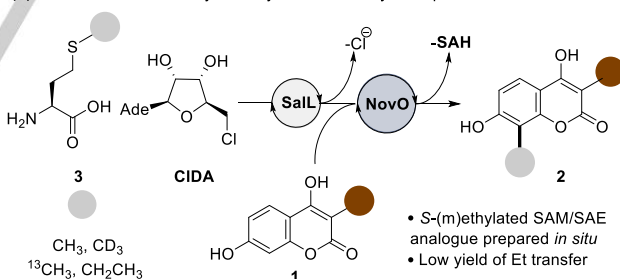
Traditional synthetic approaches have typically involved using Friedel-Crafts,<sup>[8]</sup> radical-based methods,<sup>[9, 10]</sup> and more recently, transition-metal catalyzed activation of C(sp<sup>2</sup>)-H bonds.<sup>[11-13]</sup> However, obtaining regiospecificity, particularly when this is required at a late-stage in a synthetic workflow, is an enduring challenge.<sup>[3]</sup> In contrast, MTases catalyze regiospecific C-methylation of biomolecules using the SAM cofactor as the corresponding methyl donor.<sup>[14-17]</sup>

The repertoire of C-methylation extends to small molecules, which opens up opportunities to tailor these enzymes as a general platform for biocatalytic C-C bond formation (Figure 1a). A representative example is NovO, which catalyzes the C-alkylation of coumarins (*e.g.* 1), and forms a key step in the biosynthesis of Novobiocin.<sup>[18-20]</sup> A hallmark of NovO is its substrate promiscuity (*e.g.*, 1) and the ability to utilize S-alkylated analogues of SAM to form products such as 2 (Figure 1a).<sup>[20, 21]</sup>

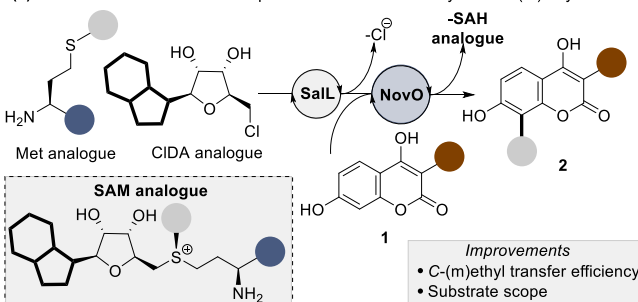
(a) Previous work: C-alkylation using SAM analogues prepared by chemical synthesis (Gruber-Khadjawi)



(b) Previous work: C-methylation by a tandem enzymatic process



(c) This work: Cofactor-directed optimization of tandem enzymatic C-(m)ethylation



**Figure 1.** Biocatalytic C-alkylation using (a) S-alkylated SAM analogues, and (b) a tandem enzymatic process. (c) Enhancement of yield and substrate scope of C-(m)ethylation by modifications to the cofactor scaffold. Ade = adenine.

- [a] Mr I.J.W. McKean and Prof. G.A. Burley\*  
Department of Pure and Applied Chemistry  
University of Strathclyde  
298 Cathedral Street, Glasgow, U.K. G1 1XL.  
E-mail: glenn.burley@strath.ac.uk
- [b] Prof. P.A. Hoskisson\*  
Strathclyde Institute of Pharmacy and Biomedical Sciences  
University of Strathclyde  
161 Cathedral Street, Glasgow, U.K. G4 0RE.  
Email: paul.hoskisson@strath.ac.uk
- [c] Dr J.C. Sadler and Dr L.D. Humphreys  
GlaxoSmithKline Medicines Research Centre,  
Gunnels Wood Road, Stevenage, U.K. SG12NY.
- [d] Dr A. Cuetos, Dr A. Frese and Prof. G. Grogan\*  
Department of Chemistry  
University of York  
Heslington, York, U.K. YO10 5DD.  
Email: gideon.grogan@york.ac.uk

Supporting information describing the detail experimental procedures can be found by following the link at the end of the document.

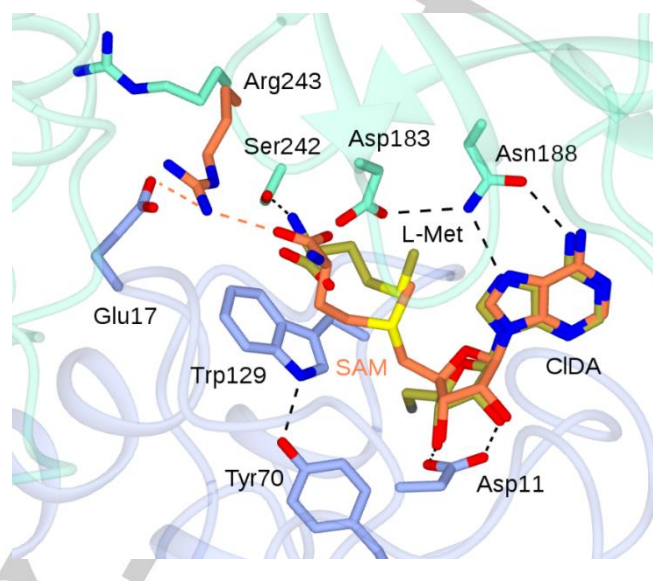
One limitation of this process is the need to prepare these cofactors by chemical synthesis, which is laborious, low yielding and produces both epimers at the sulfur centre.<sup>[22–26]</sup> Additionally, SAM analogues are inherently unstable in buffered solution ( $t_{1/2}$  942 min for SAM at pH 8).<sup>[27, 28]</sup> A more step- and atom-efficient strategy is to couple cofactor formation with C-alkyl transfer.<sup>[15, 29, 30]</sup> One example of this one-pot process is the generation of cofactor analogues *in situ* from either CIDA or ATP and (m)ethionine,<sup>[15, 29–33]</sup> followed by C-(m)ethyl transfer catalyzed by a MTase (Figure 1b).<sup>[34]</sup> CIDA is a shelf-stable, atom-economical adenosine source for such a process catalyzed by SalL compared to ATP, which is a substrate for SAM production by methionine adenosyltransferase (MAT).<sup>[15, 27, 29, 35, 36]</sup>

Although in-depth knowledge of the substrate promiscuity of C-MTases has been garnered from structural and mutagenesis studies,<sup>[17, 19, 20, 37]</sup> little is known about how the structural features of the SAM cofactor itself influences the yield and scope of C-alkylation.<sup>[14, 30]</sup> Herein, we showcase a method to address these limitations by strategic modifications to SAM and S-adenosyl ethionine (SAE, Figure 1c).

An earlier structural study of SalL in complex with CIDA and methionine revealed a solvent-exposed channel into the active site.<sup>[38–40]</sup> To explore this in more detail, we obtained two structures of wild-type SalL with SAM and chloride (6RYZ, 1.50 Å), and with CIDA alone (6RZ2, 1.77 Å; Figure 2, Table S1). One significant difference in our structures compared to those obtained previously was a rotation of the sidechain of Arg243, from the solvent exposed exterior of the protein to the interior of the active site, enabling the formation of electrostatic interactions between Arg243 and the carboxylate of SAM (Figure 2, S1), and the side chain of Glu17 from the adjacent monomer. No associated changes in the solvent exposed channel were observed. These structures were then used as a guide for the preparation of point mutants in order to explore the roles of specific residues in catalysis. Phe186Leu, Trp129Phe, Asp183Glu, Trp190Ala, Val12Met and Tyr70Met displayed a reduced level of activity relative to wildtype SalL (Table S2, Figure S2). The Phe186Leu mutant was able to form SAM and SAM analogues, albeit in slightly poorer conversions relative to the wildtype (Figure S15). In contrast, enzymatic activity was abolished in the Asp183Ala, Asn188Ala, Phe186Ala and Phe228Ile/Ala mutants. This suggests that  $\pi$ -stacking between Phe228 and the adenine nucleobase, the electrostatic interaction between the Met carboxylate and Asp183, and H-bonding to the Hoogsteen face of the adenine nucleobase (Asn188) are essential for catalysis.

Further supporting evidence for the importance of Asp183 for catalysis was observed when a tetrazole carboxylic acid bioisostere of methionine was used (Figure 3a). The use of <sup>tet</sup>Met<sup>[27]</sup> resulted in 99% conversion to **4c**, despite slower reaction kinetics relative to SAM formation (**4a**, Figure 3b, Table

S3, Figures S8–12, S14). However, an increase in steric bulk at the sulfur center *i.e.*, by replacing Met with L-ethionine formed SAE (**4b**) albeit in lower conversion (41%) relative to SAM.



**Figure 2.** WT SalL in complex with CIDA and L-Met (PDB 2Q6I<sup>[31]</sup>) superimposed with WT SalL in complex with SAM (6RYZ, this study). Neighbouring monomers and amino acid side-chains of 2Q6I are shown in cyan and light blue. Arg243 and SAM (6RYZ, coral) illustrate relocation of this side chain to form new ionic interactions (coral) with the SAM carboxylate and the side chain of Glu17.

Replacing adenine with 7-deazaadenine (**4d**) resulted in only 10% conversion (Figure 3c),<sup>[27]</sup> whereas a hypoxanthine nucleobase did not form **4e**. This suggests that the interaction between the N7 of adenine and Asn188 is critical for the catalytic function of SalL. High conversions to **4f–j** were observed using analogues containing modifications to the 2- and 6-position of adenine. Combining 2,6-diamino or 2-chloro-6-aminoadenine modifications with <sup>tet</sup>Met produced **4k** and **4l** in > 99% and 41% conversion, respectively (Figure 3d). Although no formation of **4m** was observed when <sup>tet</sup>ethionine was used, SAE analogues **4n** and **4o** were formed in 78% and 37%, respectively. Finally, no cofactor products were formed using CIDA substrates lacking either 2'/3' ribose hydroxyl groups.

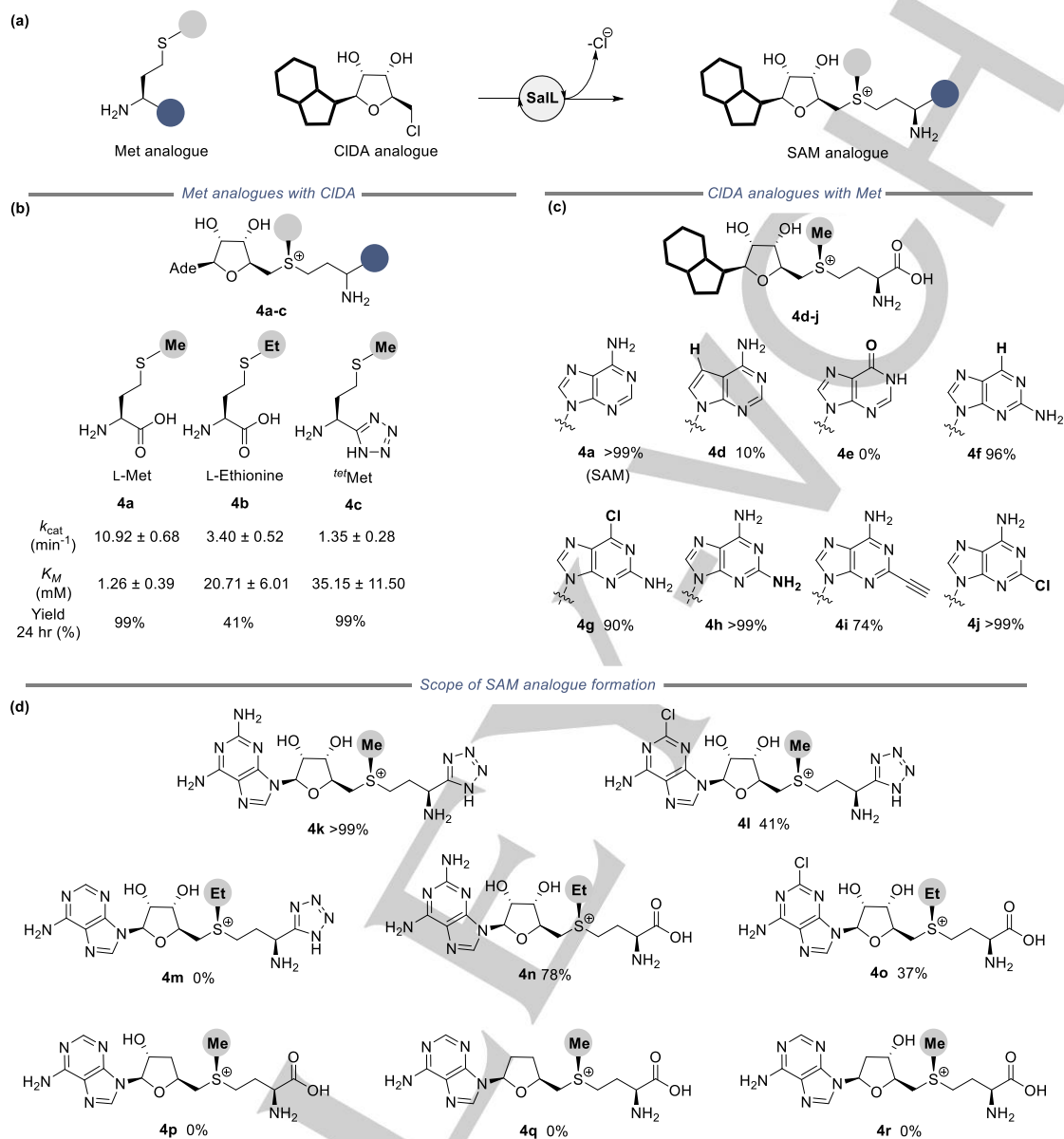
Inspection of the crystal structure of NovO in complex with S-adenosylhomocysteine (SAH) revealed the presence of a hydrophobic cleft with a volume of ~ 21 Å<sup>3</sup>.<sup>[18]</sup> This is in the exact location of the 2-position of the adenine nucleobase (Figure 4, S3). We surmised that SAM/SAE cofactors bearing modifications of complementary steric volume at this position would also be substrates for NovO. Our tandem enzymatic process using purified SalL and NovO in the presence of stoichiometric amounts of CIDA analogue, L-Met and coumarin (**5**) indeed demonstrated the enhanced conversion of methylated

coumarin (**5a**) via the *in situ* formation of modified SAM to SAM (Figure S70; Table S5).

analog

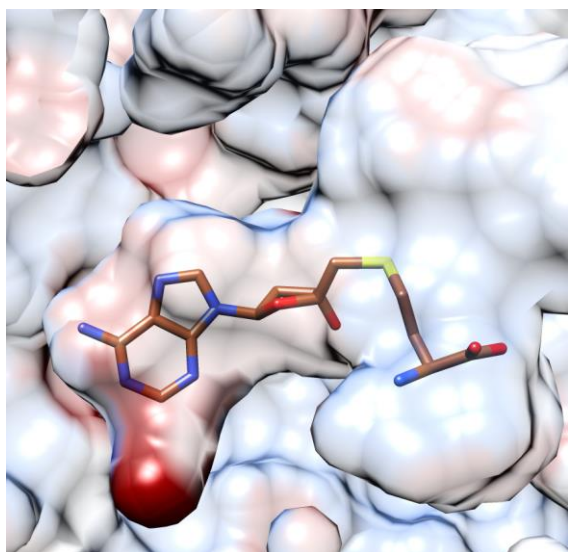
ues

relative

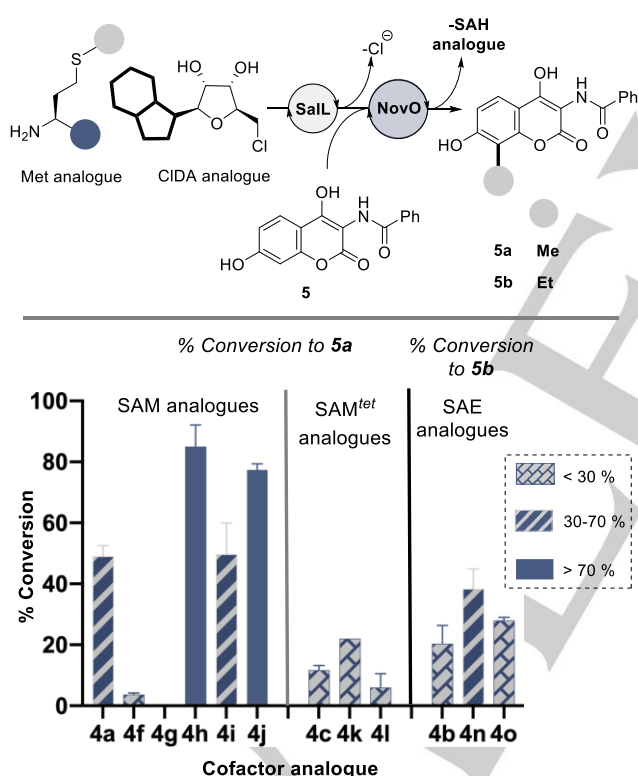


**Figure 3.** (a) Formation of SAM/SAE analogues catalyzed by SalL. (b) Reaction kinetics and % conversions using modified Met analogues. Substrate scope of cofactor synthesis incorporating (c) nucleobase and (d) a combination of nucleobase and amino acid modifications. Assay conditions: CIDA/CIDA analogue (400  $\mu\text{M}$ ), L-Met/L-Ethionine (2.00 mM), SalL (2.10  $\mu\text{M}$ ), DTT (1.00 mM) and BSA (1.00 mg/mL), potassium phosphate buffer (100 mM, pH 6.8), 24 h, 37  $^{\circ}\text{C}$ . % conversions determined by RP-HPLC using a ratio of the peak area (254 nm) of the CIDA analogue to SAM/SAE analogue and 5'-methylthioadenosine (or analogue).<sup>[27]</sup>





**Figure 4.** Crystal structure of NovO in complex with SAH highlighting the 2-position of adenine projecting towards a hydrophobic cleft (red. PDB: 5MGZ).



**Figure 5.** C-(m)ethylation of 5 catalyzed by NovO using modified cofactors.

For example, using analogues **4h** or **4j** formed *in situ*, enhanced the conversion to **5a** from 17% (using SAM) to 53% (**4h**) and 39% (**4j**) was achieved. Further enhancement was achieved using an excess of CIDA analogues (2 equiv) and L-Met (10 equiv.). In this instance, the conversion of **5** to **5a** improved to 85% (**4h**) and 77% (**4j**) relative to 49% when SAM was generated *in situ* (Figure 5). As identified in an earlier study,

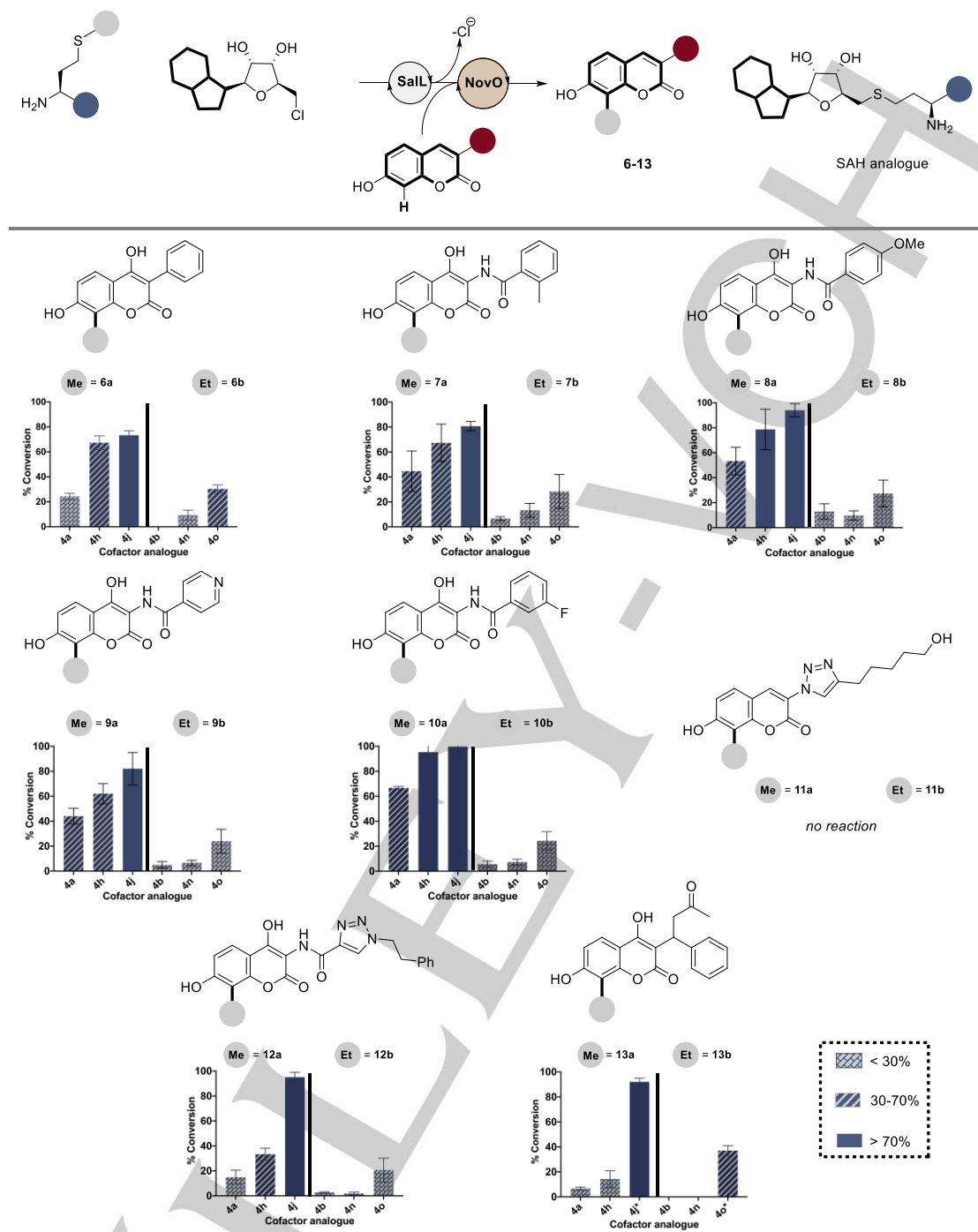
MTAN was added to the reaction mixture in order to degrade the SAH analogue, which inhibits NovO.<sup>[34]</sup> The 2-modified alkyne cofactor **4i** displayed comparable conversion (**5a**, 50%) to SAM, whereas the formation of the 2-amino-6-chloro analogue (**4g**) *in situ* did not form **5a**. Tetrazole analogues **4c**, **4k** and **4l** produced significantly lower amounts of **5a** relative to SAM. Enhanced levels of C-ethylation of **5** was also observed, producing **5b** from **4n** (39%) and **4o** (25%) relative to only 24% when SAE was used. Exploration of the wider scope of methylating (**5a-13a**) and ethylating (**5b-13b**) a suite of 3-substituted coumarins (**5-13**) exemplified the superiority of using nucleobase-modified SAM/SAE analogues (Figure 6).<sup>[34]</sup> In almost all examples, the 2-amino and 2-chloro modified cofactors out-performed SAM in methylating **5-13**. One exception was triazole **11**, in which no C-(m)ethylation was observed using any of the cofactors tested.

Finally, the ability of our tandem enzymatic process to (m)ethylate coumarin scaffolds with known biological activity and clinical relevance was explored. The core of coumarin **12a** is a precursor to a known inhibitor of Hsp90,<sup>[41]</sup> and is being pursued as an anti-cancer therapy,<sup>[42]</sup> whilst **13a** is a metabolite of warfarin.<sup>[43]</sup> C-methylation was almost quantitative, producing **12a** (95% conversion; 23% isolated yield) and **13a** (92%) when **4j** was used compared to only 15% (**12a**) and 7% (**13a**) conversion using SAM. Ethylation of both substrates produced **12b** and **13b** in 21% and 37% respectively. In contrast, **12b** was formed in only trace amounts (3%), whereas no ethylated product (**13b**) was formed using SAE.

In summary, we have established a new biocatalytic strategy which enhances the yield and substrate scope of small molecule C-(m)ethylation by incorporating nucleobase modifications within the SAM cofactor. Key to the success of this approach is the compatibility of SalL and NovO to couple *in situ* generation of SAM/SAE cofactor analogues (SalL) with C-(m)ethyl transfer (NovO). We envisage that blending directed evolution strategies with cofactor analogue mapping, and new strategies to recycle the SAH product formed by C-alkylation<sup>[15, 44]</sup> will provide new opportunities to identify enzyme variants with wider substrate promiscuity.

## Acknowledgements

I.J.W.M and G.A.B. thank the Biotechnology and Biological Sciences Research Council (BBSRC) for funding (BB/M016366/1; BB/N016378/1). J.C.S. thanks GlaxoSmithKline (GSK) and the University of Strathclyde for an industrial PhD studentship. A.C. was funded by the BBSRC (BB/P005578/1). A.F. was funded by a studentship from GSK. We thank Dr Johan Turkenburg and Mr Sam Hart for assistance with X-ray data collection and the Diamond Light Source for access to beamlines (mx-9948). Finally, we thank Dr Allan J.B. Watson for his suggestion to explore warfarin as a NovO substrate.



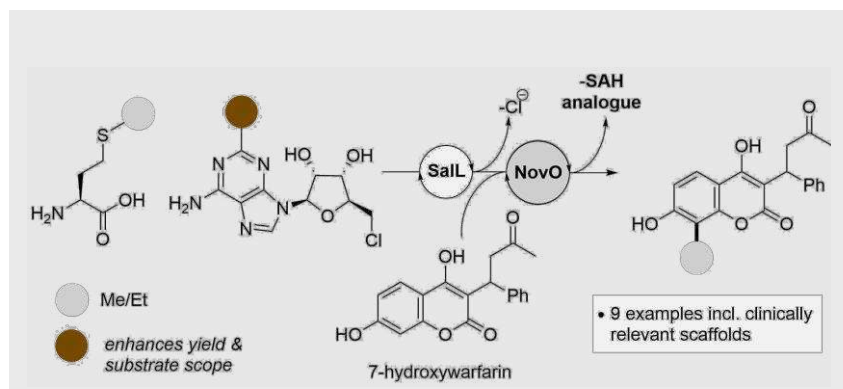
**Figure 6.** Substrate scope of C-(m)ethylation of coumarins **6-13**. CIDA/CIDA analogue (400  $\mu$ M), L-Met/L-ethionine (2.00 mM), coumarin (200  $\mu$ M), SalL (2.10  $\mu$ M), DTT (1.00 mM) and BSA (1.00 mg/mL), potassium phosphate buffer (100 mM, pH 6.8), 24 h, 37  $^{\circ}$ C then NovO (9.38  $\mu$ M) and MTAN (132 nM).<sup>[18, 34]</sup> \*Optimized conditions for **13a/13b** with **4j/4o**: 2-Cl-CIDA 1.60 mM, L-Met/L-ethionine (8.00 mM), 7-hydroxywarfarin (200  $\mu$ M), SalL (4.20  $\mu$ M), DTT (4.00 mM) and BSA (1.00 mg/mL), potassium phosphate buffer (100 mM, pH 6.8) 24 h, 37  $^{\circ}$ C then NovO (42.6  $\mu$ M) and MTAN (528 nM). % conversions were determined by RP-HPLC using a ratio of the peak area at 300 nm of the coumarin starting material to the product.<sup>[18, 34]</sup>

**Keywords:** alkylation • methyltransferase  
• S-adenosylmethionine • biocatalysis • coumarin

## References

- [1] L. D. Moore, T. Le, G. Fan, *Neuropsychopharmacology* 2012, **38**, 23.
- [2] E. M. Harcourt, A. M. Kietrys, E. T. Kool, *Nature* 2017, **541**, 339.
- [3] T. Cernak, K. D. Dykstra, S. Tyagarajan, P. Vachal, S. W. Krska, *Chem. Soc. Rev.* 2016, **45**, 546-576.
- [4] A.-W. Struck, M. L. Thompson, L. S. Wong, J. Micklefield, *ChemBioChem* 2012, **13**, 2642-2655.
- [5] H. Schönher, T. Cernak, *Angew. Chemie Int. Ed.* 2013, **52**, 12256-12267.
- [6] Y. Chen, *Chem. Eur. J.* 2019, **25**, 3405-3439.
- [7] J. Boström, D. G. Brown, R. J. Young, G. M. Keserü, *Nat. Rev. Drug Discov.* 2018, **17**, 709.
- [8] M. Rueping, B. J. Nachtsheim, *Beilstein J. Org. Chem.* 2010, **6**, 6.
- [9] J. Gui, Q. Zhou, C.-M. Pan, Y. Yabe, A. C. Burns, M. R. Collins, M. A. Ornelas, Y. Ishihara, P. S. Baran, *J. Am. Chem. Soc.* 2014, **136**, 4853-4856.
- [10] Q. Huang, S. Z. Zard, *Org. Lett.* 2018, **20**, 1413-1416.
- [11] X. Chen, K. M. Engle, D.-H. Wang, J.-Q. Yu, *Angew. Chemie Int. Ed.* 2009, **48**, 5094-5115.
- [12] J. Li, K. Korvorapun, S. De Sarkar, T. Rogge, D. J. Burns, S. Warratz, L. Ackermann, *Nat. Commun.* 2017, **8**, 15430.
- [13] G. Yan, A. J. Borah, L. Wang, M. Yang, *Adv. Synth. Catal.* 2015, **357**, 1333-1350.
- [14] M. R. Bennett, S. A. Shepherd, V. A. Cronin, J. Micklefield, *Curr. Opin. Chem. Biol.* 2017, **37**, 97-106.
- [15] S. Mordhorst, J. Siegrist, M. Müller, M. Richter, J. N. Andexer, *Angew. Chemie Int. Ed.* 2017, **56**, 4037-4041.
- [16] M. Tomkuvienė, M. Mickutė, G. Vilkaitis, S. Klimašauskas, *Curr. Opin. Biotech.* 2019, **55**, 114-123.
- [17] T. Pavkov-Keller, K. Steiner, M. Faber, M. Teng, H. Schwab, M. Gruber-Khadjawi, K. Gruber, *PLoS One* 2017, **12**, e0171056.
- [18] J. C. Sadler, C.-w. H. Chung, J. E. Mosley, G. A. Burley, L. D. Humphreys, *ACS Chem. Biol.* 2017, **12**, 374-379.
- [19] M. Pacholec, J. Tao, C. T. Walsh, *Biochemistry* 2005, **44**, 14969-14976.
- [20] H. Stecher, M. Teng, B. J. Ueberbacher, P. Remler, H. Schwab, H. Griengl, M. Gruber-Khadjawi, *Angew. Chemie Int. Ed.* 2009, **48**, 9546-9548.
- [21] M. Teng, H. Stecher, L. Offner, K. Plasch, F. Anderl, H. Weber, H. Schwab, M. Gruber-Khadjawi, *ChemCatChem* 2016, **8**, 1354-1360.
- [22] G. Lukinavičius, V. Lapienė, Z. Staševskij, C. Dalhoff, E. Weinhold, S. Klimašauskas, *J. Am. Chem. Soc.* 2007, **129**, 2758-2759.
- [23] J. Zhang, Y. G. Zheng, *ACS Chem. Biol.* 2016, **11**, 583-597.
- [24] K. Islam, Y. Chen, H. Wu, I. R. Bothwell, G. J. Blum, H. Zeng, A. Dong, W. Zheng, J. Min, H. Deng, M. Luo, *Proc. Natl. Acad. Sci. U.S.A.* 2013, **110**, 16778-16783.
- [25] W. Peters, S. Willnow, M. Duisken, H. Kleine, T. Macherey, K. E. Duncan, D. W. Litchfield, B. Lüscher, E. Weinhold, *Angew. Chemie Int. Ed.* 2010, **49**, 5170-5173.
- [26] C. Dalhoff, G. Lukinavičius, S. Klimašauskas, E. Weinhold, *Nat. Chem. Biol.* 2005, **2**, 31.
- [27] T. D. Huber, F. Wang, S. Singh, B. R. Johnson, J. Zhang, M. Sunkara, S. G. Van Lanen, A. J. Morris, G. N. Phillips, J. S. Thorson, *ACS Chem. Biol.* 2016, **11**, 2484-2491.
- [28] T. D. Huber, B. R. Johnson, J. Zhang, J. S. Thorson, *Curr. Opin. Biotech.* 2016, **42**, 189-197.
- [29] J. M. Lipson, M. Thomsen, B. S. Moore, R. P. Clausen, J. J. La Clair, M. D. Burkart, *ChemBioChem* 2013, **14**, 950-953.
- [30] C. Vranken, A. Fin, P. Tufar, J. Hofkens, M. D. Burkart, Y. Tor, *Org. Biomol. Chem.* 2016, **14**, 6189-6192.
- [31] A. S. Eustáquio, F. Pojer, J. P. Noel, B. S. Moore, *Nat. Chem. Biol.* 2007, **4**, 69.
- [32] F. Muttach, A. Rentmeister, *Angew. Chemie Int. Ed.* 2016, **55**, 1917-1920.
- [33] B. J. C. Law, A.-W. Struck, M. R. Bennett, B. Wilkinson, J. Micklefield, *Chem. Sci.* 2015, **6**, 2885-2892.
- [34] J. C. Sadler, L. D. Humphreys, R. Snajdrova, G. A. Burley, *ChemBioChem* 2017, **18**, 992-995.
- [35] S. Singh, J. Zhang, T. D. Huber, M. Sunkara, K. Hurley, R. D. Goff, G. Wang, W. Zhang, C. Liu, J. Rohr, S. G. Van Lanen, A. J. Morris, J. S. Thorson, *Angew. Chemie Int. Ed.* 2014, **53**, 3965-3969.
- [36] B. J. C. Law, M. R. Bennett, M. L. Thompson, C. Levi, S. A. Shepherd, D. Leys, J. Micklefield, *Angew. Chemie Int. Ed.* 2016, **55**, 2683-2687.
- [37] A. Gutmann, M. Schiller, M. Gruber-Khadjawi, B. Nidetzky, *Org. Biomol. Chem.* 2017, **15**, 7917-7924.
- [38] W. L. Yeo, X. Chew, D. J. Smith, K. P. Chan, H. Sun, H. Zhao, Y. H. Lim, E. L. Ang, *Chem. Commun.* 2017, **53**, 2559-2562.
- [39] S. Thompson, S. A. McMahon, J. H. Naismith, D. O'Hagan, *Bioorg. Chem.* 2016, **64**, 37-41.
- [40] S. Thompson, Q. Zhang, M. Onega, S. McMahon, I. Fleming, S. Ashworth, J. H. Naismith, J. Passchier, D. O'Hagan, *Angew. Chemie Int. Ed.* 2014, **53**, 8913-8918.
- [41] X. M. Yu, G. Shen, L. Neckers, H. Blake, J. Holzbeierlein, B. Cronk, B. S. Blagg, *J. Am. Chem. Soc.* 2005, **127**, 12778-12779.
- [42] J. Zhao, H. Zhao, J. A. Hall, D. Brown, E. Brandes, J. Bazzill, P. T. Grogan, C. Subramanian, G. Vielhauer, M. S. Cohen, B. S. J. Blagg, *MedChemComm* 2014, **5**, 1317-1323.
- [43] A. E. Rettie, K. R. Korzekwa, K. L. Kunze, R. F. Lawrence, A. C. Eddy, T. Aoyama, H. V. Gelboin, F. J. Gonzalez, W. F. Trager, *Chem. Res. Toxicol.* 1992, **5**, 54-59.
- [44] C. Liao, F. P. Seebeck, *Nat. Catal.* 2019, **2**, 696-701.

## RESEARCH ARTICLE



Iain J. W. McKean, Joanna C. Sadler, Anibal Cuetos, Amina Frese, Luke D. Humphreys, Gideon Grogan,\* Paul A. Hoskisson\* & Glenn A. Burley\*

**S-Adenosyl Methionine Cofactor Modifications Enhance the Biocatalytic Repertoire of Small Molecule C-Alkylation**

- [a] Mr I.J.W McKean and Prof. Glenn A. Burley\*  
Department of Pure and Applied Chemistry  
University of Strathclyde  
298 Cathedral Street, Glasgow, U.K. G1 1XL.  
E-mail: glenn.burley@strath.ac.uk
- [b] Prof. Paul A. Hoskisson\*  
Strathclyde Institute of Pharmacy and Biomedical Sciences  
University of Strathclyde  
161 Cathedral Street, Glasgow, U.K. G4 0RE.  
Email: paul.hoskisson@strath.ac.uk
- [c] Dr J.C. Sadler and Dr L.D. Humphreys  
GlaxoSmithKline Medicines Research Centre,  
Gunns Wood Road, Stevenage, U.K. SG12NY.
- [d] Dr A Cuetos, Dr A. Frese and Prof. G. Grogan  
Department of Chemistry  
University of York  
Heslington, York, U.K. YO10 5DD.  
Email: gideon.grogan@york.ac.uk

Supporting information describing the detail experimental procedures can be found by following the link at the end of the document.